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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/812,764	LIEW, CHOONG-CHIN	
	Examiner	Art Unit	
	Juliet C. Switzer	1634	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *23 October 2007*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *52-54 and 56-80* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *52-54 and 56-79* is/are rejected.

7)☒ Claim(s) *80* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

1. This action is written in response to applicant's correspondence received 10/23/07. Claims 52-54 and 56-57 have been amended, claims 49-51 and 55 have been canceled, and claims 58-80 have been added. Claims 52-54, and 56-80 are pending are examined herein. Applicant's amendments and arguments have been thoroughly reviewed, but are not persuasive to place the claims in condition for allowance for the reasons that follow. Any rejections not reiterated in this action have been withdrawn. The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action. **This action is FINAL.**

Claim Objections

2. Claim 80 is objected to under 37 CFR 1.75(c) as being in improper form because a multiple dependent claim cannot depend from a previous multiple dependent claim. See MPEP § 608.01(n). Accordingly, the claim 80 has not been further treated on the merits.

Double Patenting

3. The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory obviousness-type double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent either is shown to be commonly owned with this application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement.

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

4. Claims 52-54, and 56-79 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 17-35 of copending Application No. 10/980850. Although the conflicting claims are not identical, they are not patentably distinct from each other because the claims of the copending application recite methods for diagnosing or prognosing liver cancer by determining the level of RNA transcripts expressed in blood from one or more biomarkers of Table 1 or Table 2, comparing to individuals not having liver cancer or having liver cancer, wherein differential expression or the same expression indicates the presence of liver cancer. Though the claims do not particularly require CLK1, CLK1 is one of the genes listed in Table 1. It would have been prima facie obvious to one of ordinary skill in the art to practice the claimed invention in the 10/980850 application with any or all of the genes recited in the claims, including CLK1. One would have been motivated by the express suggestion in the claims to use one or more of the biomarkers of Table 1.

This is a provisional obviousness-type double patenting rejection because the conflicting claims have not in fact been patented.

Claim Rejections - 35 USC § 112

5. Claims 64, 65, 67, 69, 70, 71, 72, 73, 74, 75, 77, 78 and 79 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the

application was filed, had possession of the claimed invention. This is a rejection for new matter.

Claim 64 appears to have new matter. The specification does not provide basis for a claim which broadly states that any time a test subject's RNA expression of CLK1 is "lower" than the expression of healthy control subjects that the subject is a candidate for having liver cancer. Regarding the expression of CLK1, the specification provides only one very specific teaching, while this claim encompasses a broad genus of "lower" expression values. Table 3X teaches that the ratio of expression in patients with liver cancer samples relative to control samples is 0.61, indicating that in the tested samples, CLK1 was expressed, on average at a 0.61 times less level in patients with liver cancer versus healthy controls. The specification teaches that the samples included four patients with liver cancer and three "control" individuals. Table 3Y teaches that this result is significant p=0.0001786. Thus, the broad statement in the claim regarding classifying the subject as a candidate for liver cancer if the RNA level "is lower" appears to be new matter.

Claim 65 appears to have new matter. The specification does not provide basis for the range "at least 0.6 times lower than that of control subjects." As discussed, the specification teaches only a single example where there was an observed ratio of 0.61 between patients having liver cancer and healthy controls. There is no value for the range limit 0.6 nor for the range of values lower than 0.61.

Claim 67 appears to have new matter. The specification does not provide basis for the particular combination of observations required for the claim, namely that CLK1 expression in a test subject is "at least 0.6 times lower" with a "p value of <0.05." The limitation "at least 0.6

times lower" has been previously discussed. Further, "with a p value of <0.05" does not appear to have basis in the context of differential expression of CLK1 in a test subject versus healthy subjects. Claim 69 also includes this recitation and is rejected for having the same new matter. In a similar fashion, claim 75 is rejected for new matter.

Claim 70 has new matter because it recites that the test subject is a candidate for liver cancer if the expression is "0.61 times higher" than the control subjects classified as healthy control subjects. There is no basis for classifying a subject as a candidate for liver cancer if the CLK1 expression is HIGHER than healthy controls.

Claim 79 has new matter because it recites that if the test subject expression of CLK1 is **higher** relative to healthy control subjects the resulting classification is that the test subject expression is classified with expression of subjects having liver cancer. There is no basis in the specification for classifying expression with liver cancer expression if the expression is HIGHER than healthy controls. Likewise, there is no basis for the recitation that a statistically **lower** expression relative to liver cancer controls results in classification with healthy subjects.

In claims 71, 72, 73, 74, and 77, the limitation that the blood samples "comprises leukocytes which a have not been fractionated into cell types" is new matter. Such a recitation includes, for example, testing a blood sample where the red blood cells and the white blood cells have been separated, and also includes, the testing of whole blood RNA. There is clearly basis for the latter, but not the former. Applicant asserts in the remarks that this claim limitation finds clear support in the specification, including figure 5C which shows standardized fractions of leukocytes. However, these are not leukocytes that have not been fractionated into cell types, as they have clearly been fractionated into cell types. While RNA levels have been determined in

each of the fractions, this is not basis for the negative limitation "have not been fractionated into cell types." There is no discussion or example in the specification of the testing of RNA in blood samples which comprise leukocytes which have not been fractionated into cell types. Applicant has attempted to present a claim which excludes a particular process step from a method (that is, fractionating the leukocytes) and then provides basis for the exclusion of the step in a method where the opposite occurred. This is not sufficient basis for the claim limitation because there is nothing in the specification that suggests applicant contemplated the exclusion of a step of fractionating leukocytes into cell types. Therefore, the claims are rejected for having new matter.

All claims which depend from the specifically discussed claims are rejected for having new matter because of their dependency from the specifically enumerated claims.

Claim Rejections - 35 USC § 102

6. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.
>
> (e) the invention was described in (1) an application for patent, published under section 122(b), by another filed in the United States before the invention by the applicant for patent or (2) a patent granted on an application for patent by another filed in the United States before the invention by the applicant for patent, except that an international application filed under the treaty defined in section 351(a) shall have the effects for purposes of this subsection of an application filed in the United States only if the international application designated the United States and was published under Article 21(2) of such treaty in the English language.

7. Claims 58, 52, 53, 54, 57, 60, 61, 62, 63 , 71, 72, and 73 are rejected under 35 U.S.C. 102(e) as being anticipated by Cocks et al.

Cocks et al. teach methods for analyzing a sample using a collection of genes implicated in blood cell biology, and the collection includes CLK1 (throughout; see SEQ ID NO: 699 in Table 1).

Cocks et al. teach a method for analyzing body fluid samples, including blood samples (Col. 10, lines 54-58), wherein RNA is isolated from the samples, the target polynucleotides are reverse transcribed into cDNA, a DNA is amplified from that cDNA (Col. 11, line 1 and following) and the cDNA is then hybridized to a collection of polynucleotides which include CLK1 (Col. 12-13 and throughout). Thus, Cocks et al. teach a method for detecting expression of CLK1 in a human test subject comprising detecting RNA encoded by said gene in a blood sample of said test subject, using an oligonucleotide of predetermined sequence which is specific for RNA encoded by CLK1.

The method taught by Cocks et al. includes quantifying a level of RNA encoded by said gene in a sample (Col. 13, lines 4-25) and comparing said level of RNA to a quantified level of control (Col. 13, lines 11-20 and Col. 6 lines 54-65). Control subjects taught by Cocks et al. include healthy patients (Col. 6, beginning at line 55).

Cocks et al. teach quantifying relative to "normalization genes" which are housekeeping genes within the scope of the claim (see Col. 13).

Claims 71-73 are rejected because Cocks et al. teach measuring expression in blood samples. In order to do so, a whole blood sample inherently would have to be taken from an individual. While Cocks et al. are silent as to how the RNA will be isolated or if the cells will be fractionated, the instant claims are drawn using "comprising" language and allow for additional manipulations of the whole blood sample which are not expressly set forth in the claims.

8. Claims 58, 60, 53, 57, 71 and 72 are rejected under 35 U.S.C. 102(a) and 102(b) as being anticipated by Steidl et al. Blood (November 15, 2001), Vol. 98, No. 11, Part 1, p. 551a.

This application is a CIP of a series of parent applications. The earliest filings, namely the 09/477,148 filing teach that CLK1 was found as an expressed gene in blood, but this filing does not provide any further discussion or disclosure regarding this gene in particular, nor the differential expression of this gene in any particular disease state, and particularly not liver cancer. There is not sufficient disclosure in the parent application to enable the use of the instantly claimed methods for at least the reasons discussed in this office action, particularly since the disclosure in the parent applications is even less than that in the instant application. The Steidl et al. reference, is thus available as prior art under 102(a) and 102(b). In the event that applicant is able to establish adequate support for the claimed invention under 112 1st paragraph in an application filed prior to the date of availability of the instant reference, the rejection will be withdrawn.

Steidl et al. teach the isolation of human CD34+ cells from peripheral blood, and detection of RNA encoding CLK1 in the blood cells using a microarray which had thereupon an oligonucleotide of predetermined sequence which is specific for cDNA complementary to said gene, and Steidl et al. quantify the level of RNA in the sample.

Claim 71 and 72 are rejected because Steidl et al. measure expression in a whole blood sample. While Steidl et al. teach subsequent separation of particular cells out of the sample, the instant claims are drawn using "comprising" language and allow for additional manipulations of the whole blood sample which are not expressly set forth in the claims.

Claim Rejections - 35 USC § 103

9. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

10. Claims 59, 52, 53, 54, 57, 60, 61, 62, 63 , 71, 72, and 73 are rejected under 35 U.S.C. 103(a) as being unpatentable over Cocks et al. (US 6607879) in view of Chenchik et al. (US 5,994,076).

The teachings of Cocks et al. have been discussed previously in this office action.

Regarding claim 59, Cocks et al. do not teach a method which includes producing an amplification product from RNA encoded by said gene using primers specific for only for RNA encoded by said gene and/or for cDNA complementary to RNA encoded by said gene.

However, at the time the invention was made, it was known to use gene specific primers to produce amplification products prior to hybridization with predefined arrays, as taught by Chenchik et al. (throughout; Col. 11).

It would have been prima facie obvious to one of ordinary skill in the art to have modified the invention taught by Cocks et al. so as to have used gene specific primers to amplify target sequences prior to hybridization with a microarray. In this case, all of the claimed elements were known in the prior art and one skilled in the art could have combined the known elements as claimed to provide a predictable result, namely the production of probe hybridization molecules particularly amplified to hybridize to the array taught by Cocks et al.

Claims 52, 53, 54, 57, 60, 61, 62, 63 , 71, 72, and 73 are included in this 103 to address the embodiment wherein they depend from claim 59.

11. Claim 56 is rejected under 35 U.S.C. 103(a) as being unpatentable over Cocks et al. in view of Bassam et al. (Australasian Biotechnology, Volume 6, Number 5, pages 285-294).

The teachings of Cocks et al. are provided above.

Cocks et al. do not teach determining gene expression using quantitative PCR.

Bassam et al. discuss methods of fully automated real time quantitative PCR.

Because both Cocks et al. and Bassam et al. teach methods for quantifying expression of genes in biological samples, it would have been obvious to one of ordinary skill in the art to substitute one method for the other to achieve the predictable result of determining gene expression levels in a biological sample.

12. Claims 52, 53, 54, 57, 58, 60, 61, 62, 63, 71, 72, 73, 75, 76, 77, and 78 are rejected under 35 U.S.C. 103(a) as being unpatentable over Xu et al. (PNAS, Vol. 98, No. 26, pages 15089-15094) in view of Cocks et al. and Sharma et al. (WO 98/49342, as cited in IDS).

Xu et al. teach a method for detecting expression of a variety of human genes in human test subjects diagnosed of having liver comprising detecting RNA encoded by said gene in said subject using an oligonucleotide of predetermined sequence which is specific for RNA encoded by said gene and/or for cDNA complementary to RNA encoded by said gene.

In particular Xu et al. teach using DNA chip expression analysis using microarrays.

Xu et al. do not teach detecting applying their analysis to the gene expression in a blood sample, and in particular do not teach detecting CLK1 in a blood sample.

Cocks et al. teach methods for analyzing a sample using a collection of genes implicated in blood cell biology, and the collection includes CLK1 (throughout; see SEQ ID NO: 699 in Table 1).

Cocks et al. teach a method for analyzing body fluid samples, including blood samples (Col. 10, lines 54-58), wherein RNA is isolated from the samples, the target polynucleotides are reverse transcribed into cDNA, a DNA is amplified from that cDNA (Col. 11, line 1 and following) and the cDNA is then hybridized to a collection of polynucleotides which include CLK1 (Col. 12-13 and throughout). Thus, Cocks et al. teach a method for detecting expression of CLK1 in a human test subject comprising detecting RNA encoded by said gene in a blood sample of said test subject, using an oligonucleotide of predetermined sequence which is specific for RNA encoded by CLK1.

The method taught by Cocks et al. includes quantifying a level of RNA encoded by said gene in a sample (Col. 13, lines 4-25) and comparing said level of RNA to a quantified level of control (Col. 13, lines 11-20 and Col. 6 lines 54-65). Control subjects taught by Cocks et al. include healthy patients (Col. 6, beginning at line 55).

Cocks et al. teach quantifying relative to "normalization genes" which are housekeeping genes within the scope of the claim (see Col. 13).

Cocks et al. do not particularly and clearly point out that the RNA tested is total blood RNA, an embodiment set forth in many of the claims in this application.

Sharma et al. teach that from the very early stages of diseases the whole organism response to the changed condition (p. 10, 4th full ¶). In light of this, Sharma et al. teach a method for identifying a marker useful for diagnosing a disease comprising the steps of detecting

the presence of RNA in an unfractionated sample of whole blood from each of one or more subjects having said disease and quantifying a level of said RNA in said sample. Namely, Sharma et al. teach the preparation of gene transcript patterns beginning with extraction of mRNA from tissues, cells or body parts of an individual or organism which has a disease or condition (p. 7, final ¶, p. 12, 1st ¶), and particularly teach the isolation of mRNA from unfractionated whole blood samples, where unfractionated is interpreted as meaning that the cell types within blood were not separated from one another (p. 35, section 5.1.1). Sharma et al. teach quantifying the level of expression and determining a difference between the quantified level in the sample from the diseased subject and a similarly quantified level of genes of control RNA from an unfractionated sample of whole blood from each of one or more first control subjects (p. 5, step (d); p. 15, first full ¶; p. 18, step (f); p. 11, final ¶). Sharma et al. teach that these methods are carried out by producing amplification products from RNA extracted from an unfractionated sample of whole blood (p. 18 and p. 35, Example 5). Sharma et al. specifically suggest that this method can be applied to the study of a variety of different types of cancer (p 6, 2nd ¶).

Therefore, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have modified the methods taught by Xu et al. so as to have additionally tested the blood of the patients having liver cancer and the healthy control samples using the microarray taught by Cocks et al., and in particular to have completed this testing on total blood RNA. One would have been so motivated by the express teachings of Sharma et al. that disease exerts a global effect on individuals and that this effect can be measured by gene expression in the blood. The identification of markers for disease in the blood suggests a

potential minimally invasive method to detect liver cancer. One would have been motivated to use the microarray analysis taught by Cocks et al. since they teach that their array has potential use in the identification of genes differentially expressed in cancers.

At the time the invention was made, in differential expression assays using microarrays, expression statistical significance indicating a difference between two expression levels was commonly met if the p value was <0.05 (p. 19). These ranges include the values set forth in claims 75 and 76, and thus if they were observed when practicing the method taught by Xu et al. in view of Cocks et al. and Sharma they would have been be considered to indicate differentially expressed genes by one of ordinary skill in the art at the time the invention was made.

Claim Rejections - 35 USC § 112

13. Claims 52-54 and 56-74 and 79 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

Nature of the invention

Claim 58 is drawn to a method for detecting expression of CDC-like kinase 1 (CLK1) in a human "test subject." Claims which depend from claim 58 set forth that the detected expression is quantified and compared to quantified level of control RNA encoded by said gene in blood samples of control subjects. Listed control subjects include healthy subjects, subjects having liver cancer and subjects that do not have liver cancer. Further dependent claims set forth steps of classifying or identifying the test subject as being a candidate for having liver cancer

depending on the outcome of the comparing steps. Thus, it appears that the intended use of claim 58 and those that depend from claim 58 is for classifying or identifying the test subject as being a candidate for having liver cancer.

Independent claim 69 sets forth a method for screening a human test subject for having liver cancer and includes similar detection, quantification, and comparing steps, reciting that a test subject is a candidate for having liver cancer if said level of RNA encoded by said gene in said blood sample of the test subject is "at least 0.6 times lower" than that of said healthy control subjects with a p value <0.05. Claim 70 is similar, but recites that the subject is a candidate for having liver cancer if the level of RNA encoded by said gene is 0.61 times higher than that of said control subjects classified as healthy subjects with a p value equal to 0.000178.

The nature of the invention requires the knowledge of a reliable relationship between CLK1 expression in blood and the presence of liver cancer.

In claim 79, the invention is drawn to a method a method for classifying CLK1 gene expression in a human, and sets forth steps of quantifying a level of RNA encoded by a CLK1 gene, comparing that level to a level of RNA found in blood samples from control subjects having liver cancer and also comparing it to control subjects who are healthy. The independent claim states that based on particular determinations, the classification of CLK1 gene expression results either with that of said subjects having liver cancer or with that of subjects who are healthy.

The nature of the invention requires the knowledge of a reliable association between CLK1 expression and the ability to classify a particular individual's expression with the expression of subjects having liver cancer or not having liver cancer, and further, the use of this

method requires that there is an underlying assumption that this classification is meaningful. Reading the claims in light of the specification it is clear that applicant intends to use such a classification method in order to provide a tool that is used as part of a diagnostic process, and such a use requires the knowledge of a reliable association underlying the classification. Further, the practice of the invention requires an understanding of how the presence of liver cancer effects the level of CLK1 expression in human blood in patients having liver cancer versus patients that do not have liver cancer but may have some other disorders.

Scope of the claims

Many aspects of the claims remain quite broad.

In some claims the health status of the control individuals is entirely undefined, and encompass subjects with liver cancer, healthy patients, patients with some other disease, such as hypertension, obesity, hyperlipidemia or rheumatoid arthritis.

Many claims recite that a difference is identified but do not require that the difference is statistically significant at any particular level, and so, any level of difference observed can result in classifying the test subject as a candidate for disease. These claims do not recite a level of statistical significance that is required to be reached, and so, the claims remain quite broad since no particular level is required, and the claims even encompass using different levels of statistical significance for different comparisons. The phrase "statistically significant" describes a mathematical measure of difference between groups, not a particular level of difference which is acceptable. There is no universally accepted level of "statistically significant."

Furthermore, some of the claims result in classifications that are the opposite of what is suggested by the results in table 3Y. Namely, claims 70 and 79 classify patients or expression

with liver cancer patients or expression if the level is statistically higher relative to the control subjects when the specification teaches that the CLK1 expression was 0.61 in liver cancer patients relative to healthy cancer.

Claim 58 is representative of the narrowest claims set forth in the instant claim set that sets forth relationships that are supported by the data in the specification. This claim specifically defines the control population as healthy subjects and sets forth a very particular ratio of gene expression in the test subject relative the healthy control subjects.

Teachings in the Specification/Examples

Regarding liver cancer, the specification provides example 26 wherein gene expression profiles of blood samples from individuals having liver cancer were compared with normal individuals, that is healthy patients. The specification teaches that 1,475 genes were identified as being differentially expressed, and regarding the instant claims, table 3X provides a list of these genes (Example 26). CLK1 is among the genes.

Table 3X teaches that the ratio of expression in patients with liver cancer samples relative to control samples is 0.61, indicating that in the tested samples, CLK1 was expressed, on average at a 0.61 times less level in patients with liver cancer versus healthy controls. The specification teaches that the samples included four patients with Liver cancer and three "control" individuals. Table 3Y teaches that this result is significant $p=0.0001786$.

The specification also teaches that CLK1 is differentially expressed ($p<0.05$) in the blood of patients having hypertension, obesity, hyperlipidemia and rheumatoid arthritis versus normal controls (tables 3A, 3B, 3E, 3H, and 3M). For each of these diseases, the specification is silent as to the nature of the differential expression. The specification fails to teach whether the

difference is an up regulation or down regulation and the specification fails to teach the magnitude of the difference.

The claims are inclusive of claims which classify a subject as "a candidate for liver cancer" based on any observation of "lower" CLK1 expression relative to healthy controls, yet the specification teaches that 0.61 fold difference in expression was observed.

The specification does not provide data to support the assertion in claims 70 and 79, namely that if a test subject expresses higher CLK1 relative to a healthy control subject they are properly classified with liver cancer patients. As discussed, the specification expressly teaches the opposite.

Claim 54 is limited to a case where the control subjects do not have liver cancer, but they could still have any other possible disease or condition. For example, the claims are inclusive of control subjects that have hypertension or obesity. For this embodiment of the claims, the specification does not provide information about an essential aspect of the invention, namely, evidence that there is a difference in expression of the CLK1 gene between these two populations.

Furthermore, though the specification teaches that this gene is differentially expressed in liver cancer patients versus healthy patients, the specification teaches this is true for thousands of genes. There is no guidance or analysis of data in the specification to suggest that this gene in particular is sufficient to classify a patient as a candidate for liver cancer, as is instantly claimed. To the contrary, the specification clearly indicates that CLK1 is differentially expressed in test versus control patients for a variety of conditions.

State of the Prior Art and Level of Unpredictability

Observing differences in expression between two populations is a highly unpredictable endeavor. While the instant specification teaches that CLK1 is differentially expressed in a population of liver cancer patients versus control subjects, the specification does not establish that any particular level of expression of CLK1 (relative level or raw level) is sufficient to reliably classify expression with liver cancer expression or to reliably identify a patient as a candidate for having liver cancer.

The expression of genes in example 26 was tested by hybridization of samples to a microarray that contains genetic information for tens of thousands of genes. This technology area is highly unpredictable, and as a result significant guidance is required to practice inventions data obtained from such experiments. Lee (Clinical Chemistry, 47:8, 1350-1352 (2001)) teaches that despite the technical accuracy of individual observations on an array, these data "are much more prone to numerous false-positive findings fundamentally because of (a) an extremely large number of observations and (b) a very wide dynamic range of gene expression values obtained from gene chip experiments." In view of these unpredictable aspects of applying such data, Lee teaches that replication is necessary to begin to screen out false positive results. There is no replication in the instant specification.

Chenchik et al. characterize CLK1 as a gene that is a "stress gene" meaning that it modulates cellular response to stressors (¶0019 and Example 2). Thus differential expression of this gene in the blood of patients may simply indicate stress in the patient. This exemplifies that it is highly unpredictable what can be reasonable concluded based on the blood sample of a test patient, since differential expression versus a control could indicate some other disorder or phenotype is present, whether that is obesity, hypertension, rheumatoid arthritis, hyperlipidemia,

some other cancer phenotype, some other liver disease or some other disease which has not yet been analyzed.

It is unknown and unpredictable whether CLK1 is differentially expressed in patients having cancers other than liver cancer or having liver disorders other than liver cancer compared to healthy controls. Without this knowledge, it is not possible to conclude that differential expression of CLK1 in the blood of a test individual is sufficient to classify a test subject as a candidate for having liver cancer.

The instant specification has not established that all difference, no matter the magnitude, relative to any control subjects or even relative to a healthy control subject is indicative of liver cancer, or sufficient to categorize a test subject's expression of CLK1 as those with liver cancer. Furthermore, the specification has not shown that all expression at a level statistically the same as that observed in a population of patients having liver cancer is sufficient to conclude that liver cancer is present. In fact, it is unclear if this is a fair conclusion given the fact that CLK1 is also demonstrated to be differentially expressed in other phenotypes such as hyperlipidemia and rheumatoid arthritis. It is entirely unpredictable if this is also the case with other diseases. It is not known under what circumstances the result observed in the instantly examined control and test populations would be repeatable, as the results have not been validated. The specification has not shown that it is appropriate to classify a patient's expression with those having liver cancer if the expression of CLK1 is higher than controls who have liver cancer. All of these inquiries are particularly important in this case since the claims suggest or recite that these classifications or identifications are important.

Further, some of the claims of the instant application set forth the comparison of the gene expression in a single individual versus as few as two other individuals, and they set forth that a comparing gene expression between the two is useful to suggest the presence liver cancer. Neither the specification nor the claims set forth a threshold of difference between an individual's expression and the control samples expression of CLK1 in the blood that would be sufficient to conclude that the difference in gene expression between a test individual and any type control group is "indicative of" the recited liver cancer. Because some of the claims encompass any level of altered gene expression, it is relevant to point out that the art of Cheung et al (2003) teaches that there is natural variation in gene expression among different individuals. The reference teaches an assessment of natural variation of gene expression in lymphoblastoid cells in humans, and analyzes the variation of expression data among individuals and within individuals (replicates) (p.422, last paragraph; Fig 1). The data indicates that, for example, expression of ACTG2 in 35 individuals varied by a factor of 17; and that in expression of the 40 genes with the highest variance ratios, the highest and lowest values differed by a factor of 2.4 or greater (Fig 3). It is thus unpredictable as to whether or not any level of altered gene expression is indicative of a liver cancer or the absence of liver cancer.

The unpredictability of correlating gene expression level to any phenotypic quality is taught in the post-filing art of Wu (2001). Wu teaches that gene expression data, such as microarray data, must be interpreted in the context of other biological knowledge, involving various types of 'post genomics' informatics, including gene networks, gene pathways, and gene ontologies (p.53, left col.). The reference indicates that many factors may be influential to the outcome of data analysis, and teaches that expression data can be interpreted in many ways. The

conclusions that can be drawn from a given set of data depend heavily on the particular choice of data analysis. Much of the data analysis depends on such low-level considerations as normalization and such basic assumptions as normality (p.63 - Discussion). The art of Newton et al (2001) further teaches the difficulty in applying gene expression results. Newton et al. teaches that a basic statistical problem is determining when the measured differential expression is likely to reflect a real biological shift in gene expression, and replication of data is critical to validation (p.38, third full paragraph). There is no replication of data in the instant specification.

Quantity of Experimentation

The instant specification does not provide enabling support for the practice of a single embodiment within the claimed invention. Claims 70 and 79 the claims recite features that are opposite of the example in the specification. Further, although the specification teaches there are differences in CLK1 levels in a liver cancer population versus a control patient population, and the specification teaches that for this population the difference is a 0.61 fold decrease, the specification does not support the assertion in the claims that observing such an decrease relative to any and all control populations of 2 or more individual is sufficient to suggest liver cancer is present. Thus, given the lack of teaching in the specification and the highly unpredictable nature of the technology, an extensive amount of work would be required to practice the claimed invention.

In order to practice the claimed invention, one would have to undertake an extensive amount of experimentation in a highly unpredictable technology area. One would have to begin by validating the results observed in the instant specification in a separate and larger population of healthy and liver cancer patients, in view of the established level of unpredictability in this

technology area. One would have to further complete similar analysis for other diseases and conditions and control populations versus healthy controls and versus liver cancer controls in order to attempt to establish when and if analysis of CLK1 expression is sufficient to suggest liver cancer is present. For example, consider the comparison of a test result and a control population of healthy individuals. If the test result is different from the level of expression observed in the healthy control group, does this mean liver cancer is suggested? How different from the average level of expression of healthy individuals would the test result have to be to indicate liver cancer- is a 0.61 fold difference required or a higher or lower threshold? Would any difference, up or down regulation be indicative of liver cancer? Or could one result indicate liver cancer and one a different disease such as hyperlipidemia or rheumatoid arthritis or cellular stress? Is CLK1 expressed in the blood of individuals with a disease other than those taught in the specification? Is this expression also diagnostic of other cancers, liver diseases or other disorders entirely unrelated to liver cancer? In order to reliably use a method for detecting liver cancer, one would first have to answer at least these questions. One would also, however, have to carry out this testing for validation, for it is possible that the result observed in the instant specification is intrinsic to the very small cohort of patients evaluated in applicant's study. Further, one would have to undertake experimentation to determine difference thresholds required to determine that a patient has or does not have a disease.

Conclusion

The claims include methods which encompass the detection in blood of the expression of CLK1 in a test subject and comparing this expression to control subjects, wherein the wherein the results are used to "classify the expression" or to suggest that an individual is a candidate for

having liver cancer. The identification of gene differential expression/disease indication relationships is a highly unpredictable endeavor, requiring extensive experimentation. The specification provides minimal guidance. In light of the factors discussed, therefore, it is concluded that it would require undue experimentation to practice the claimed invention.

Although some of claims are drawn to a method of "detecting expression" or "classifying expression," and not to diagnosis or identifying increased likelihood of disease or the like, it is critical to understand how the classification can be used in order use the claimed invention. In this case, the specification does not provide sufficient guidance as to how to use the detecting or classification methods, or in other words what is the meaning of classifying expression "with that of subjects having" liver cancer or with subjects who are healthy? While one could do the method steps as written, thus satisfying the "how to make" aspect of 112 1st paragraph, the specification does not provide sufficient disclosure to satisfy the how to use aspect of the requirement.

Claim 58 represents a very narrow embodiment of the claimed invention, but still is based on data that is not replicated. As discussed in the rejection, it is established that the technology on which the instant claims is based is a highly unpredictable technology, and in the face of such a high level of unpredictability, replication is necessary before results can be considered sufficient to support claims directed at classifying the gene expression of an individual test subject. Therefore, even this claim, after having considered all of the factors set forth in this rejection, lacks proper enablement.

Response to Remarks

Applicant traverses the rejection for obviousness type double patenting because the conflicting application is under a restriction requirement, and election may obviate the instant double patenting rejection. As of the writing of this office action, this is not the case. The rejection is maintained. Applicant also points out that if the obvious double patenting rejection is the only remaining rejection in an earlier-filed application it should be withdrawn. This is not the case in this earlier filed application and so the rejection is maintained.

The rejection under 112 1st paragraph has been amended to address the amended and newly added claims. Applicant traverses the rejection insofar is it applies to the pending claims, beginning on page 13 of the response.

Applicant states that the instant claims recite three clearly defined sets of controls. Not all claims are so limited. In addition,."patients that do not have liver cancer" is sufficiently broad so as to encompass patients with all of the other diseases discussed in the rejection.

Applicant states that the newly added claims specify a direction and a level of difference in CLK1 expression to be detected. Applicant refers specifically to claim 75 which is not included in the enablement rejection. Further, it is noted that the limitation quoted as being in claim 75 on page 14 of the response is not actually in claim 75. Nonetheless, as noted in the enablement rejection, not all of the claims are so narrowly limited, and some claims set forth relationships that are the opposite of those set forth in the specification. Further, even if the claims were all narrowly limited to mirror the relationships set forth in the specification, in a technology area of such a high degree of unpredictability, external validation would be extremely useful to establish the reliability of the observed relationships. In this case, very small sample sizes were used to establish the relationships set forth in the claims.

Applicant states that the fact that CLK1 is a stress gene and the disclosure of many other differentially expressed genes between healthy patients and those with liver cancer does not detract from the gene in a biomarker for liver cancer, but provides no further argument or evidence to support this assertion. These are two factors in a compilation of factors that lead to the conclusion of undue experimentation. Applicant points out that the specification discloses that RNA encoded by the CLK1 gene is 0.61 times lower than that of healthy subjects with a p value = 0.0001786. This is acknowledged and discussed in the rejection. The specification also teaches this gene is differentially expressed in a number of other diseases but provides no guidance as to how. It is highly unpredictable as to whether that expression is the same or different as for other diseases.

Applicant contends on page 15 of the response that one of skill in the art can reasonably predict with statistical significance the probability that a patient may be a candidate for liver cancer based on the teachings of the specification since the specification teaches the statistically significant correlation between levels of CLK1 RNA in blood of a diseased versus healthy controls. At the time the invention was made, the need for validation of such a result obtained by a microarray analysis was well understood by one of skill in this technology. The issues regarding the unpredictability of this technology are discussed in detail in this office action.

Applicant states that Chenchik does not teach whether CLK1 functions as a stress gene in blood and that it cannot be concluded that the differential expression of CLK1 in blood may simply indicate stress. The examiner did not make such a conclusion, but used the reference to point out that it is unpredictable what can be concluded based on the expression levels of this

gene in blood. Further, applicant's own specification supports this position by demonstrating that this gene is differentially expressed in a variety of disease states.

Applicant disagrees with Wu that expression data needs to be interpreted in view of other biological knowledge. Wu was relied upon for much more than this simple statement. Wu discusses at length many of the factors that make gene expression analysis unpredictable. Applicant's statement that "differential gene expression which is reproducible, and is correlated with the state of health or disease of the individual does not necessarily result directly in the state of the disease of the individual" is attorney argument which is not supported by evidence on the record. Even if the changes are a result of downstream effects of the pathogenic process, they are related to the state of disease in the individual. Applicant points out that certain prostate markers were used as biomarkers without an understanding of their function. The examiner is not trying to require an understanding of CLK1 in liver cancer or any other disease, nor does Wu suggest that such is necessary. The examiner is looking to the specification for adequate guidance for making and using an invention in a highly unpredictable field of endeavor.

Applicant states that the results of Cheung et al. cannot be reliably extrapolated to primary blood samples since Cheung et al. are using cultured cell lines. However, this is irrelevant to the point of Cheung et al. which is that among individuals (in this case cell lines) there is natural variability in gene expression for any particular gene. Attorney arguments are not sufficient to establish that this biological fact is not the case.

Applicant states that the extension of the experiments as outlined in the specification to additional individuals is merely routine (page 17). However, this is not persuasive given the highly unpredictable nature of this technology area, as previously discussed.

Conclusion

14. No claim is allowed.

15. Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

16. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Juliet C Switzer whose telephone number is (571) 272-0753. The examiner can normally be reached on Monday, Tuesday, or Wednesday, from 9:00 AM until 4:30 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ram Shukla can be reached by calling (571) 272-0735.

The fax phone numbers for the organization where this application or proceeding is

assigned are (571) 273-8300. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (571)272-0507.

Patent applicants with problems or questions regarding electronic images that can be viewed in the Patent Application Information Retrieval system (PAIR) can now contact the USPTO's Patent Electronic Business Center (Patent EBC) for assistance. Representatives are available to answer your questions daily from 6 am to midnight (EST). The toll free number is (866) 217-9197. When calling please have your application serial or patent number, the type of document you are having an image problem with, the number of pages and the specific nature of the problem. The Patent Electronic Business Center will notify applicants of the resolution of the problem within 5-7 business days. Applicants can also check PAIR to confirm that the problem has been corrected. The USPTO's Patent Electronic Business Center is a complete service center supporting all patent business on the Internet. The USPTO's PAIR system provides Internet-based access to patent application status and history information. It also enables applicants to view the scanned images of their own application file folder(s) as well as general patent information available to the public.

For all other customer support, please call the USPTO Call Center (UCC) at 800-786-9199.

/Juliet C. Switzer/
Primary Examiner
Art Unit 1634

January 2, 2008